Short Communication

# Antioxidative study of polysaccharides extracted from red (*Kappaphycus alvarezii*), green (*Kappaphycus striatus*) and brown (*Padina gymnospora*) marine macroalgae/seaweed

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**Abstract**
Sterile and fresh tissues of three marine macroalgae red, green and brown (*Kappaphycus alvarezii, Kappaphycus striatus* and *Padina gymnospora*) collected from Malaysia east costal seas were compared for the antioxidants and polysaccharide composition of sugars as well as the active components. Results obtained showed that polysaccharides isolated from *Kappaphycus alvarezii, Kappaphycus striatus* and *Padina gymnospora*) can be used as a source of natural antioxidant compounds as they possess antioxidant potential in which the *Padina gymnospora* showed 15.56 ± 0.12 mg/mL to be the best antioxidants among all the polysaccharides studied. The hot water extraction method is effective in isolating polysaccharides from studied seaweeds. The GC–MS analysis revealed that there is presence of chemical compounds such as furfural was 25.53% in *Kappaphycus alvarezii* and 21.04% in *Kappaphycus striatus* also *Padina gymnospora* incorporates n- Hexadecanoic acid about 26.31% in seaweed polysaccharides that contribute to their antioxidant activities. Further studies can be done on determining the seaweed species that are available abundantly with the best source of natural antioxidant compounds.

**Keywords** DPPH · ABTS · Seaweed · GC–MS

## 1 Introduction

Nowadays, there is an increasing demand by the pharmaceutical industries to develop natural antioxidant. Biomedical and pharmaceutical field are in an emerging interest of seaweeds research due to their various contents of bioactive compounds. Seaweed are a large diverse group of eukaryotic, macroscopic, photosynthetic and marine organism. Seaweeds can produce variety of bioactive components for the benefits of humans [2, 3]. Seaweeds are rich and a renewable source of structurally and functionally unique polysaccharides which is important in the application ranging from pharmaceuticals to food industry [15].

Seaweed also contain secondary metabolite which its function is to defence against free radicals, herbivores, fouling organisms and pathogens. It is also play role in the reproduction, protection from UV radiation and as allelopathic agents. The example of secondary metabolite is, terpenes, acetogenins, alkaloids and polyphenolics. In the south east coastal region, marine brown algae (Phaeophyceae), *Sargassum wightii* which belong to the family Sargassaceae major found in that region. [7]. Presence of the secondary metabolite in the seaweed such as polyphenols,

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polysaccharide, β carotene and α tocopherol make them able to produce metal and metal oxide nanoparticles [21].

Seaweeds can be classified into three main groups depending on their pigments and colouration which are the red marine algae (Rhodophyta), brown marine algae (Phaeophyceae) and green marine algae (Chlorophyta). Seaweeds contain high number of polysaccharides normally found at the cell wall matrix [6, 8]. Seaweeds have distinct polysaccharides, which chemical structures of the polysaccharides depends on the type and cell structures of seaweeds [25]. Souza et al. [20] claimed that the seaweed-derived polysaccharides are effective and non-toxic antioxidants. Brown seaweeds have many different types of polysaccharides such as alginate, laminarans and also sulfated fucans known as fucoidan that show high potential in therapeutics [19].

The commonly known sulfated polysaccharides isolated from red seaweed are sulfated galactans. Brown seaweeds contain sulfated polysaccharides which are homo-polysaccharide and hetero-polysaccharide containing α-L-sulfated fucose are called fucan and fucoidan, respectively. Besides, green seaweed contains sulfated polysaccharide which normally heteropolysaccharides containing galactose, mannose, xylose, glucose, arabinose and glucuronic acid [9]. The extraction of important components such as ulvan from green seaweed become crucial for its application in pharmaceutical field and food industry [26].

This research investigation encompasses isolation of polysaccharide from three different species of seaweeds (*Kappaphycus alvarezii*, *Kappaphycus striatus* and *Padina gymnospora*) were using hot water extraction method and the determination of its antioxidant activity with antioxidant assays and finally GC–MS analysis showed that there is presence of chemical compounds in seaweed polysaccharides for future natural antioxidant production in various pharmaceutical applications.

## 2 Materials and methods

### 2.1 Collection, identification, and processing of seaweeds

The seaweeds were collected at Pekan, East coastal region, Malaysia during low tide. They sample of seaweeds were collected and identified. Among the seaweeds, *Kappaphycus alvarezii*, *Kappaphycus striatus* and *Padina gymnospora* were used for the identification of polysaccharides. The collected seaweeds were washed in seawater to get rid of macroscopic epiphytes, sand particles and other extraneous matter and rinsed with distilled water. After that, the seaweeds (red, brown and green) were airdried in shady place and ground into fine powder for the use of further analysis [24].

### 2.2 Extraction and deproteinization of polysaccharide from seaweed

The extraction method was done by following the method of Shao et al. [18] with some minor modifications. 100 g of powdered red seaweed was extracted in 1 L of distilled water at 100 °C for 3 h using reflux apparatus. The slurry was filtered using filter paper and the filtrate was collected. The extraction was repeated three times and the filtrates were combined, then centrifuged at 4500 rpm for 10 min. The supernatant was decanted and concentrated to about 200 mL under reduced pressure using rotary evaporator. Then, the supernatant was precipitated with addition of threefold volume (about 600 mL) of 95% ethanol. The mixture was allowed to stand for overnight at room temperature. The precipitate was collected by vacuum filtration and washed with absolute ethanol. The ethanol was then removed by rotary evaporation. Finally, the crude polysaccharides were obtained by freeze-drying method. The same extraction procedures were repeated for powdered brown and green seaweeds. The freeze-dried seaweed powder was dissolved in distilled water with (100% w/v). The concentrated solution of seaweed sulfated polysaccharide was adjusted to pH 3 with 10% TCA solution and kept overnight. The sample was centrifuged at 5,000 rpm for 10 min. and the supernatant was collected to obtain the deproteinized solution. This procedure was repeated 2 to 3 times [7].

### 2.3 Determination of sugar content (Phenol–sulphuric acid method)

The sugar content of seaweed sulfated polysaccharides were determined using phenol–sulphuric acid assay following the methods of [27] with some modifications. Different concentrations of polysaccharides solutions were prepared. 3.2 mL of 80% sulphuric acid were added quickly into each tube of sulfated polysaccharide solution with different concentrations. The mixtures in the tubes were allowed to reach maximum reaction temperature for at least one minute. The temperatures of the tubes were then reduced quickly by inserting them into water bath. After that, 50 µL of phenol was added and the tubes were allowed to stand at room temperature for 30 min. Finally, the absorbance was taken at the wavelength of 480 nm.

## 2.4 Antioxidant assay

### 2.4.1 DPPH radical scavenging activity

The antioxidant activity of seaweed extract was measured with 1–1-Diphemyl-2-picryl-hydrazyl (DPPH) using the method reported by Tingting et al. [22]. 2 ml of sample solution (polysaccharide solutions) at different concentrations (0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, 1.0 mg/mL, 1.2 mg/mL and 1.4 mg/mL) was added to 2 ml 0.2 mM ethanol solution of DPPH, the reaction mixture was shaken vigorously and incubated for 30 min. in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm. The procedure mentioned above was repeated for positive control which were ascorbic acid, gallic acid and Butylated Hydroxyanisole (BHA). The blank solutions were prepared by adding 3 mL ethanol to 0.1 mL of sample solutions with different concentrations. The negative control was prepared by adding 3 mL of DPPH-ethanol solution and 0.1 mL ethanol. The ability of scavenging the DPPH radicals was calculated using the following equation:

$$\text{Scavenging effect}\,(\%) = (1 - (A_{\text{sample}} - A_{\text{blank}})/A_{\text{control}} \times 100$$

where $A_{\text{sample}}$ is absorbance of DPPH solution with the tested sample, $A_{\text{blank}}$ is the absorbance of the blank and $A_{\text{control}}$ is the absorbance of negative control.

### 2.4.2 ABTS inhibition assay

ABTS (2,2'azino bis (3-ethylbenzothiazoline-6-sulphonic-acid) diammonium salt), radical cation decolourization assay was used to determine the antioxidant activity of seaweed extract following the method claimed by Barahona et al. (2011) with some modifications. The ABTS radical stock solution was prepared by reacting equal volume of ABTS solution (dissolved 2.2208 mL ABTS assay in 5 ml water) and potassium persulfate solution (dissolved 13.2 mg potassium persulfate in 20 ml water). The stock solution was then allowed to stand in the dark at room temperature for 12–16 h before use. This ABTS radical cation solution maintained in its stable condition for at least 2 days. The ABTS radical cation solution prepared was then diluted with Phosphate Buffered Saline (PBS) to absorbance of 0.70 ± 0.02 at wavelength of 734 nm. 1 ml of the ABTS radical cation solution was added to each tube of polysaccharide sample solution, lactose and ascorbic acid standard solutions with different concentrations (0.2 mg/mL, 0.4 mg/mL, 0.6 mg/mL, 0.8 mg/mL, 1.0 mg/mL, 1.2 mg/mL and 1.4 mg/mL). The blank was prepared by adding 3 ml PBS with 0.1 ml of different concentrations as mentioned previously. The negative control was prepared by adding ABTS radical cation solution with 0.1 ml PBS. Finally, the absorbance readings of all tubes with mixtures of solutions and assays were taken at wavelength of 734 nm.

$$\text{ABTS radical scavenging activity}\,(\%) = ((Ao - A1)/Ao) \times 100$$

where Ao is Absorbance of control and A1 is Absorbance of sample.

## 2.5 Statistical analysis

All the assays were carried out in triplicate and the values were expressed in the form of mean ± standard error (SE). IBM SPSS statistic 20 was used to analyse the data obtained. One-way analysis of variance (ANOVA) was used to compare the mean values of the tail intensity. Then, the analysis continued through Turkey's multiple range tests. The statistical significance is at $p < 0.05$.

## 2.6 GC–MS characterization

The extracted polysaccharides from brown, red and green seaweeds were freeze dried. The powder was diluted in the hexane and filtered prior to send to GC analysis. The sample was separated on a 30 m × 0.25 mm ID × 0.25 µm film thickness column DB 1 ms (agilent) attached to the GC-2010 (GCM-QP 2010) SHIMADZU chromatography equipment with a flame-ionization detector and a split injector. High purity hydrogen was used as the carrier gas at a flow rate of 1 ml/min the column temperature was maintained at 200 °C and 240 °C respectively, and 1 µl sample in dichloromethane was injected through a glass-lined splitter which set at 1/10 ratio. The absorption was then read between 40 m/z and 800 m/z [24]. The results obtained were scanned with preinstalled library of standards.

# 3 Results and discussion

## 3.1 Extraction and deproteinization of seaweed polysaccharides

The identified seaweeds, Kappaphycus alvarezii, *Kappaphycus alvarezii* and *Padina gymnospora* (Fig. 1) were processed for extraction of polysaccharides. The polysaccharides were extracted from seaweeds using water extraction method with hot water at the temperature of 100 °C. Hot water instead of cold water was used in the extraction as the researchers found in their studies that hot water extraction method gave higher yield of polysaccharides than cold water extraction due to low extraction

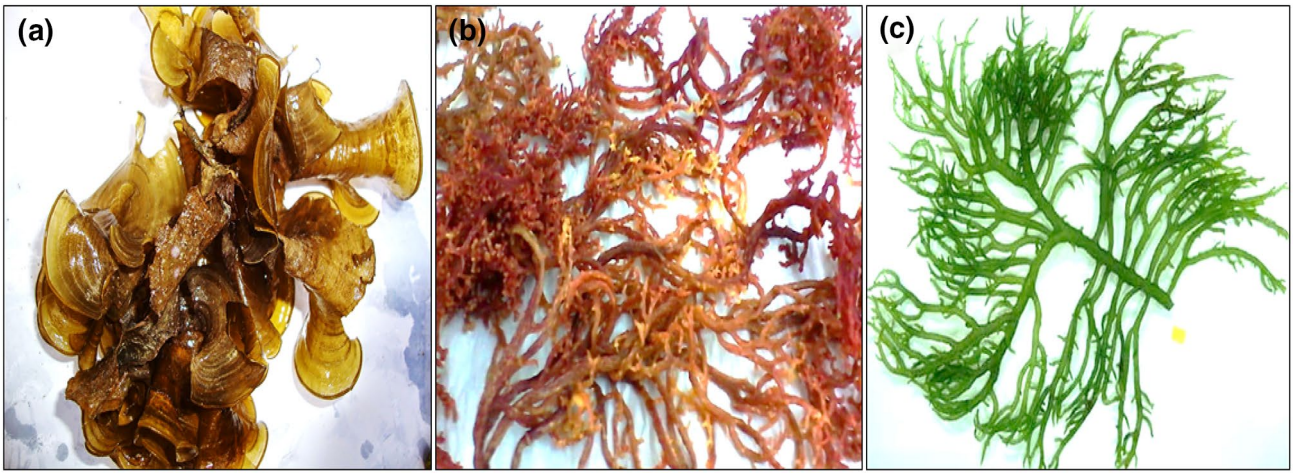


**Fig. 1** Marine seaweed samples **a** (Phaeophyceae) Brown variant (*Padina gymnospora*) **b** (Rhodophyta) Red variant (*Kappaphycus alvarezii*), and **c** Green variant (*Kappaphycus striatus*)

temperature [2, 3]. They also claimed that the research showed the extraction temperature is one of the factors which is responsible for the yield of extraction. The seaweeds were extracted under reflux using reflux apparatus as this technique prevents boiling dry of reaction vessel as vapour rapidly condensed in the condenser and the boiling can be proceeded at a constant temperature [2, 3, 20]. Besides, different methods can also be used to precipitate out polysaccharides other than using ethanol and determination of the yield of polysaccharides in order to identify the best method for precipitating polysaccharides. Besides, other extraction method of extraction such as methanol extraction, microwave and ultrasound extraction can also be carried out in order to identify the most suitable method in extracting high yield of polysaccharides from seaweeds.

According to Shao et al. [18], the extraction of seaweeds was repeated three times and all the filtrates were combined in order to maximise the yield of seaweed extracts. Then, the seaweed extracts obtained were precipitated with 95% ethanol as this concentration of ethanol showed better precipitation of polysaccharides. Finally, the polysaccharides with the removal of ethanol by rotary evaporation were freeze-dried to obtain crude polysaccharides in powder form.

The deproteinization of seaweed polysaccharides was carried out using Trichloroacetic Acid (TCA) method. Rodrigues et al. [17] claimed that hot water extraction may denature some of the proteins and may be removed by centrifugation while the remaining proteins can be removed by deproteinization using Trichloroacetic Acid (TCA) or calcium chloride ($Ca\ Cl_2$) methods. TCA method was used for deproteinization of seaweed sulfated polysaccharides as Huang et al. [7] claimed that TCA method was more effective than $Ca\ Cl_2$ method in removing protein as it was found in their study that protein content of the samples with deproteinization using $Ca\ Cl_2$ method was higher than using TCA method. The TCA method utilises principle of binding of protein cation to TCA molecules to produce an insoluble salt at isoelectric point [7].

### 3.2 Determination of sugar content (Phenol–sulphuric acid method)

The sugar content of polysaccharides was determined based on the glucose calibration curve with equation $y = 0.7199x + 1.233$ and $R^2 = 0.9888$. Table 1 shows the sugar content in 1 mg/mL of polysaccharides from brown, red and green seaweed. It can be observed from the table that the sugar content is the highest in polysaccharides from *Padina gymnospora* (42.17%), followed by polysaccharide from *Kappaphycus alvarezii* (11.78%) and the lowest sugar content found in polysaccharide from *Kappaphycus striatus* (0.63%). Pujol et al. [14] reported that the sugar content of polysaccharides varies with the species of the seaweed instead of their types. Their research showed that brown seaweeds (Phaeophyceae) which are *Stoechospermun polypodioides*, *Polycladia indica* found to contain 43%

**Table 1** Sugar content analysis of polysaccharides extracted from different species of seaweed

| Samples (Polysaccharides from different species of seaweeds) | Sugar content in 1 mg/mL of polysaccharides (mg%) |
|---|---|
| *Padina gymnospora* (brown variant) | 42.17 |
| *Kappaphycus alvarezii* (red variant) | 11.78 |
| *Kappaphycus striatus* (green variant) | 0.63 |

and 40% of sugar content respectively; whereas red seaweeds (Rhodophyta) which are *Polycladia indica*, *Gracilaria corticata* and *Scinaia hatei* contain 43%, 34% and 39% of sugar content respectively; while green seaweeds (Chlorophyta) which are *Caulerpa racemosa* contain 37% of sugar content.

### 3.3 DPPH radical scavenging activity

Figure 2 shows the comparison of DPPH radical scavenging activity between BHA (positive control) and polysaccharides from red seaweed (*Kappaphycus alvarezii*), green seaweed (*Kappaphycus striatus*) and brown seaweed (*Padina gymnospora*). It can be observed that the scavenging activity increases with an increase in the concentrations of polysaccharides. This result is in agreement with the statement by Yuling et al. [28] in which the concentration determines the scavenging activity of polysaccharides on the inhibition of the DPPH free radical. The researchers also emphasized on the importance of sulfation ratio in antioxidant ability as they found in their research that the scavenging activity of sulphated polysaccharides is higher than that of de-sulphated polysaccharides.

It can be observed from the Fig. 2 that the polysaccharide from brown seaweed (*Padina gymnospora*) showed the highest percentage of scavenging activity, followed by the scavenging activity of polysaccharides from red seaweed (*Kappaphycus alvarezii*) whereas the polysaccharide from green seaweed (*Kappaphycus striatus*) showed the lowest percentage of scavenging activity at all concentrations of polysaccharides studied. However, scavenging activity of all polysaccharides is lower than that of BHA.

Table 2 shows half maximal inhibitory concentration ($IC_{50}$) of BHA (positive control) and polysaccharides from the three species of seaweeds studied. The $IC_{50}$ of polysaccharides from *Padina gymnospora*, *Kappaphycus alvarezii* and *Kappaphycus striatus* were 0.5256 ± 0.05 mg/mL, 0.6451 ± 0.12 mg/mL and 1.522 ± 0.08 mg/mL respectively which were higher than that of BHA with $IC_{50}$ of 0.31 ± 0.03 mg/mL. The lower $IC_{50}$ values indicate higher antioxidant activity as lower concentration is required to reduce the DPPH free radical at 50%. Therefore, this study shows that the antioxidant activities of polysaccharides from *Padina gymnospora* is the highest among all the polysaccharides studied but lower than positive control while the antioxidant activity of polysaccharides from *Kappaphycus alvarezii* is higher than that of *Kappaphycus striatus*.

According to Musa and Abdullah [11], there was a significant correlation between the total phenolic content and $IC_{50}$ as they found in their research that high level of total phenolic content resulted in low $IC_{50}$ values and this shows that the polyphenolic contents present in seaweed extracts are capable of scavenging free radicals.

**Table 2** Free radical scavenging activity ($IC_{50}$) of polysaccharides from brown, red and green seaweeds using BHA as a positive control

| Samples (Polysaccharides from different species of seaweeds) | $IC_{50}$(mg/mL) |
|---|---|
| *Padina gymnospora* (brown variant) | 2.565 ± 0.02 |
| *Kappaphycus alvarezii* (red variant) | 7.859 ± 0.09 |
| *Kappaphycus striatus* (green variant) | 15.56 ± 0.12 |
| Ascorbic acid (positive control) | 0.2859 ± 0.02 |

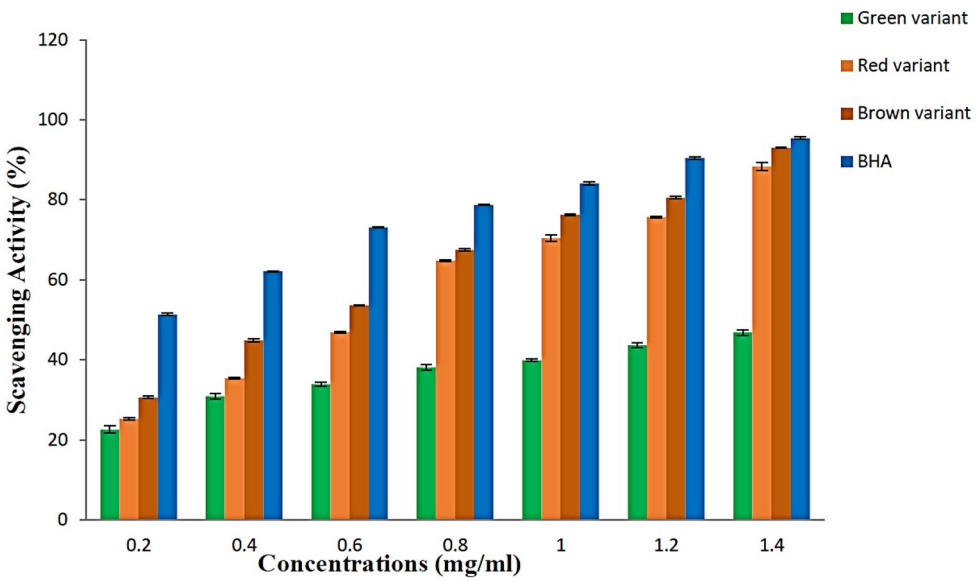


**Fig. 2** Comparison of the scavenging activity of the polysaccharides (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mg/mL) and positive control BHA

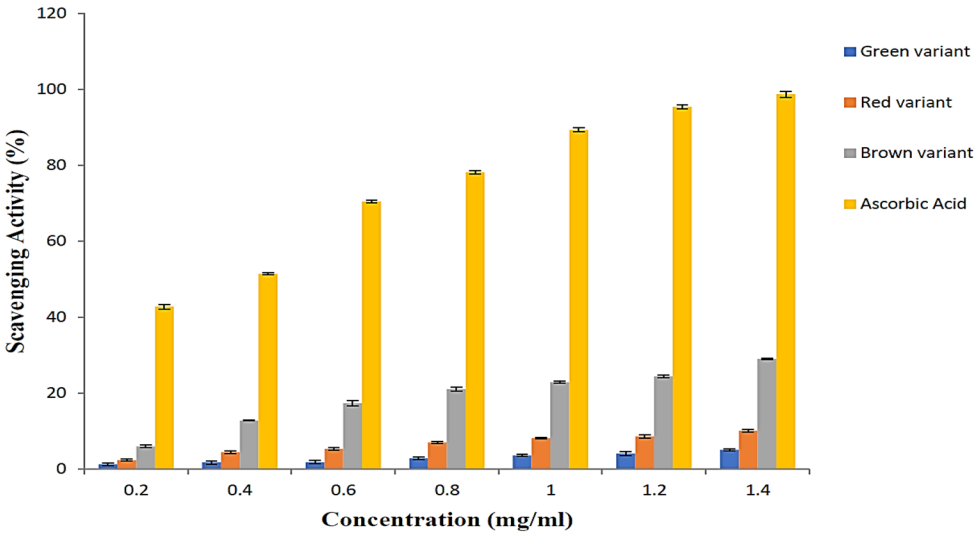


**Fig. 3** Comparison of the scavenging activity of the polysaccharides (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4 mg/mL) and positive control ascorbic acid

**Table 3** ABTS free radical activity ($IC_{50}$) of polysaccharides from brown, red and green seaweeds using ascorbic acid as positive control

| Samples (Polysaccharides from different species of seaweeds) | $IC_{50}$(mg/mL) |
|---|---|
| *Padina gymnospora* (brown seaweed) | 0.5256 ± 0.05 |
| *Kappaphycus alvarezii* (red variant) | 0.6451 ± 0.12 |
| *Kappaphycus striatus* (green variant) | 1.522 ± 0.08 |
| BHA (positive control) | 0.31 ± 0.03 |

### 3.4 ABTS inhibition assay

Figure 3 shows the comparison of ABTS radical scavenging activity between ascorbic acid (positive control) and polysaccharides from brown seaweed (*Padina gymnospora*), red seaweed (*Kappaphycus alvarezii*) and green seaweed (*Kappaphycus striatus*). It can be observed from the Fig. 3 that the scavenging activity increases with an increase in the concentrations of polysaccharides. This result is in agreement with the statement by Pawlak et al. [13], in which the free radical scavenging activity increases as the concentrations of antioxidants, flavonoids and trolox increases. The polysaccharides from brown seaweed (*Padina gymnospora*) shows the highest scavenging activity percentage among all the polysaccharides studied but lower than that of ascorbic acid, followed by polysaccharides from red seaweed (*Kappaphycus alvarezii*) and lastly the polysaccharides from green seaweed (*Kappaphycus striatus*) which show the lowest scavenging activity percentage.

Table 3 shows $IC_{50}$ of ascorbic acid (positive control) and polysaccharides from the three species of seaweeds studied. The $IC_{50}$ of polysaccharides from *Padina gymnospora*, *Kappaphycus alvarezii* and *Kappaphycus striatus* were 2.565 ± 0.02 mg/mL, 7.859 ± 0.09 mg/mL and 15.56 ± 0.12 mg/mL respectively which were higher than that of ascorbic acid with $IC_{50}$ of 0.2859 ± 0.02 mg/mL. The lower $IC_{50}$ values indicate higher antioxidant activity as lower concentrations of polysaccharides required to reduce ABTS free radical at 50%.

According to the results observed it can be said that the polysaccharide from *Padina gymnospora* is the strongest antioxidant that exhibit highest antioxidant activity, followed by polysaccharides from *Kappaphycus alvarezii* and *Kappaphycus striatus* This result is in agreement with the research result obtained by Barahona et al. [1] where the researchers found in their study that the antioxidant capacity of polysaccharides (sulphated galactan and fucoidan) from brown seaweed *Sargassum aquifolium*, *Luteimonas vadosa* respectively are higher than that of polysaccharide from red seaweed, carrageenans. The researchers also claimed that different in antioxidant capacity may be caused by the chemical structure of the polysaccharide which play an important role on the hydrogen abstraction reaction by the ABTS cation radical. They also reported that low molecular weight chitosan is effective in scavenging ABTS cation radical [16].

The natural acids that exist naturally in plant extracts may change their pH value and be resulted in affecting the estimation of antioxidant activity [4, 16]. The antioxidant activity which is achieved by deprotonation and ionization potential of the reactive functional group of antioxidants causing the reaction occurring between the examined antioxidant and the cation radical become pH dependent.

Besides, the measurement of antioxidant activity using this method is affected by the type of alcohol which is used as solvent. The researchers found that the fastest kinetic of ABTS cation radical (ABTS·+) was observed for propanol-1 and the lowest for methanol. This may be due

to the different amount of residual water content in different type of alcohol applied. The kinetics of reaction between ABTS·+ and antioxidants is highly dependent on water content in the measuring system as an increase in the water concentration in the methanolic system increases the reaction between ABTS·+ and antioxidants (Dawidowicz and Olszowy 2011; [15]).

## 3.5 GC–MS analysis

### 3.5.1 Polysaccharides from *Kappaphycus alvarezii* (Red Seaweed)

Table 4 shows the presence of some useful polysaccharide components in seaweed in which high area percentage indicates high content of particular components. Results obtained components found in sulphated polysaccharides from red seaweed in which furfural is in high content with area percentage of 25.53%. Li et al. [9] claimed that 5-Hydroxymethyfurfural and 2-Furancarboxaldehyde are common major products of carbohydrate metabolism found in plants and foods which contain carbohydrate and it had been proven in showing antioxidant potential. The researchers found in their studies that 2-furanmethanol [5], pyrazole [23] possess antioxidant properties.

### 3.5.2 Polysaccharides from *Kappaphycus striatus* (Green Seaweed)

Table 5 shows the presence of some useful polysaccharides in seaweed polysaccharides in which high area percentage indicates high content of particular components. Results obtained components found in polysaccharides from green seaweed in which furfural is in high content with area percentage of 21.04%. It had been proven from the previous research that the chemical components of polysaccharides which are furfural [9], exhibit antioxidant properties, 1H-imidazole [12], 2-Furanmethanol [5], 5-Hydroxymethyfurfural [9] and Hydrazine [27].

**Table 5** Gas chromatography analysis of polysaccharide component's present in *Kappaphycus striatus*

| Peak | Retention time | Chemical components | Area percentage |
|---|---|---|---|
| 4 | 3.452 | Furfural | 21.04 |
| 4 | 3.452 | 1H-imidazole | 21.04 |
| 11 | 7.028 | 2-Furanmethanol | 14.39 |
| 18 | 8.819 | 5-Hydroxymethyfurfural | 12.04 |
| 22 | 9.975 | Hydrazine | 4.56 |
| 27 | 18.163 | Hexadecanoic acid | 3.47 |
| 29 | 19.777 | Heptadecanoic acid | 3.31 |
| 19 | 9.157 | 2-Heptanol | 1.17 |
| 31 | 21.150 | Methyl stearate | 1.12 |

### 3.5.3 Polysaccharides from *Padina gymnospora* (Brown Seaweed)

Table 6 shows the presence of some useful polysaccharide components in brown seaweed in which high area percentage indicates high content of particular components. Results obtained showed presence some components found in polysaccharides from brown seaweed in which n- Hexadecanoic acid is in high content with area percentage of 26.31%. The Table 6 shows some chemical components found in polysaccharides from green seaweed in which n- Hexadecanoic acid is in high content with area percentage of 26.31%. The table shows thechemical components which are *n*-Hexadecanoic acid [10], Furan [2, 3], and Furfural [9].

## 4 Conclusion

Polysaccharides can be extracted from different species of seaweeds and identification of the seaweed species that give polysaccharides with best antioxidant activities. Present research investigation concluded that polysaccharides isolated from *Kappaphycus alvarezii*, *Kappaphycus striatus* and *Padina gymnospora* can be used as a source of

**Table 4** Gas chromatography analysis of polysaccharide component's present in *Kappaphycus alvarezii*

| Peak | Retention time | Chemical components | Area percentage |
|---|---|---|---|
| 10 | 7.103 | 4-Aminopyrimidine | 12.50 |
| 11 | 7.331 | Levoglucosenone | 16.62 |
| 15 | 8.081 | Nicotinyl alcohol | 9.61 |
| 3 | 3.572 | Furfural | 25.53 |
| 10 | 7.103 | 2-Furanmethanol | 12.50 |
| 18 | 9.185 | 5-Hydroxymethyfurfural | 5.65 |
| 6 | 5.048 | 2-Furancarboxaldehyde | 2.12 |
| 12 | 7.429 | 4,6-Diamino-5-pyrimidinyl hydrogen sulfate | 0.75 |
| 25 | 0.18 | 2,4,6-Triamino-5-pyrimidinyl hydrogen sulfate | 0.18 |

**Table 6** Gas chromatography analysis of polysaccharide component's present in *Padina gymnospora*

| Peak | Retention time | Chemical components | Area percentage |
|---|---|---|---|
| 5 | 18.701 | *n*-Hexadecanoic acid | 26.31 |
| 1 | 6.942 | Furan | 12.66 |
| 1 | 6.942 | Furfural | 12.66 |
| 6 | 21.551 | Octadecanoic acid | 10.96 |
| 7 | 21.894 | Pyrimidine-2,4,6-trione, 5-(1-tert -butyl-1H-pyrrol-2-ylmethylene)-1-(3-fluorophenyl) | 4.24 |
| 2 | 13.380 | 2-Furancarboxaldehyde | 3.79 |
| 4 | 18.341 | 7H-Purin-6-amine, | 7.00 |
| 8 | 23.868 | 1,1,1,5,7,7,7-Heptamethyl-3,3-bis (trimethylsiloxy) tetrasiloxane | 11.91 |

natural antioxidant compounds as they possess antioxidant potential in which the *Padina gymnospora* shown to be the best antioxidants among all the polysaccharides studied. The hot water extraction method is effective in isolating polysaccharides from *Padina gymnospora*, *Kappaphycus alvarezii* and *Kappaphycus striatus*. The GC–MS analysis showed that there is presence of chemical compounds in seaweed polysaccharides that contribute to their antioxidant activities. Large scale production of natural antioxidant compound from seaweeds can be considered as it contains many bioactive compounds that contribute to antioxidant activities. Further studies can be done on determining the seaweed species that are available abundantly with the best source of natural antioxidant compounds.

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**Declarations**

**Conflict of interest** The authors declare that they have no conflict of interest.



## References

1. Barahona T, Chandia NP, Encinas MV, Matsuhiro B, Zuniga EA (2011) Antioxidant capacity of sulfated polysaccharides from seaweeds: a kinetic approach. Food Hydrocolloids. 25(3):529–535
2. Bhuyar P, Rahim MHA, Sundararaju S, Ramaraj R, Maniam GP, Govindan N (2020) Synthesis of silver nanoparticles using marine macroalgae Padina gymnospora and its antibacterial activity towards pathogenic bacteria. Beni-Suef Univ J Basic Appl Sci 9(1):1–15
3. Bhuyar P, Rahim MH, Sundararaju S, Maniam GP, Govindan N (2020) Antioxidant and antibacterial activity of red seaweed Kappaphycus alvarezii against pathogenic bacteria. Glob J Environ Sci Manag 6(1):47–58
4. Dawidowicz AL, Olzzowy M (2013) The importance of solvent type in estimating antioxidant properties of phenolic compounds by ABTS assay. Eur Food Res Technol 465(3):558–578
5. Eskin NA, Tamir S (2006) Dictionary of nutraceuticals and functional foods. CRC Press, Boca Raton
6. Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. BioResources 56(8):158–190
7. Huang G, Cheng F, Ding X (2012) Extraction and deproteinization of cucumber polysaccharide with high scavenging ability toward superoxide anion. Afr J Biotech 11(40):9649–9853
8. Jungwirth, J. (2010). Seaweeds and human health. Retrieved from: http://www.naturespiritherbs.com/Seaweeds_and_Human_Health.pdf
9. Li MM, Wu LY, Fan M (2011) The protective role of 5-hydroxymethyl-2-furfural (5-HMF) against acute hypobaric hypoxia. Cell Stress Chaperones 18(5):529–538
10. Meechaona R, Sengpracha W, Banditpuritat J, Kawaree R, Phutdhawong W (2007) Fatty acid content and antioxidant activity of Thai bananas. Mj Int J Sci Tech 01(02):222–228
11. Musa T, Abdullah D (2013) In vitro antioxidant and enzyme inhibitory properties and phenolic composition of M. neglecta Wallr. (Malvaceae) fruit: a traditional medicinal fruit from Eastern Anatolia. Ind Crops Prod 51(3):376–380
12. Naik N, Kumar HV, Rangaswamy J, Harini ST, Umeshkumar TC (2012) Three component one pot synthesis of 5-Substituted 1-Aryl-2,3- diphenyl imidazoles: a novel class of promising antioxidants. J Appl Pharm Sci 2(11):067–074
13. Pawlak K, Bylka W, Jazurek B, Matlawska I, Sikorska M, Manikowski H, Bialek Bylka G (2009) Antioxidant activity of flavonoids of different polarity, assayed by modified ABTS cation radical decolorization and EPR technique. Acta Biologica Cracoviensia. Series Botanica 52(1):97–104

14. Pujol CA, Ray S, Ray B, Damonte EB (2012) Antiviral activity against dengue virus of diverse classes of algal sulfated polysaccharides. Int J Biol Macromol 51(4):412–416
15. Ramli ANM, Badrulzaman SZS, Hamid HA, Bhuyar P (2021) Antibacterial and antioxidative activity of the essential oil and seed extracts of Artocarpus heterophyllus for effective shelf-life enhancement of stored meat. J Food Process Preserv 45:e14993
16. Ramli ANM, Manap NWA, Bhuyar P, Azelee NIW (2020) Passion fruit (Passiflora edulis) peel powder extract and its application towards antibacterial and antioxidant activity on the preserved meat products. SN Appl Sci 2:1748
17. Rodrigues JAG, Vanderlei ESO, Bessa EF, Magalhaes FA, Paula RCM, Lima V, Benevides NMB (2011) Anticoagulant activity of a sulfated polysaccharide isolated from the green seaweed Caulerpa cupressoides. Braz Arch Biol Technol 54(4):691–700
18. Shao P, Chen M, Pei Y, Sun P (2013) In intro antioxidant activities of different sulfated polysaccharides from chlorophytan seaweeds *Ulva fasciata*. Int J Biol Macromol 59(3):295–300
19. Silva TH, Alves A, Popa EG, Reys LL, Gornes ME, Sousa RA, Silva SS, Mano JF, Reis RL (2012) Marine algae sulfated polysaccharides for tissue engineering and drug delivery approaches. Biomatter 2(4):1–12
20. Souza BWS, Cerqueira MA, Bourbon AI, Pinheiro AC, Martins JT, Teixeira JA, Coimbra MA, Vicente AA (2012) Chemical characterization and antioxidant activity of sulfated polysaccharide from the red seaweed *Gracilaria birdiae*. Glob J Pharm 27(2):287–292
21. Sundararaju S, Arumugam M, Bhuyar P (2020) Microbacterium sp. MRS-1, a potential bacterium for cobalt reduction and synthesis of less/non-toxic cobalt oxide nanoparticles (Co3O4). Beni-Suef Univ J Basic Appl Sci 9:44
22. Tingting H, Dan L, Yan C, Jun W, Shusheng W (2010) Antioxidant activity of sulphated polysaccahrides fractions extracted from *Undaria pinnitafida in vitro*. Int J Biol Macromol 46(2):193–198
23. Umesha KB, Rai KML, Nayaka MAH (2009) Antioxidant and Antimicrobial Activity of 5-methyl-2-(5-methyl-1,3-diphenyl-1H-pyrazole-4-carbonyl)-2,4-dihydro-pyrazol-3-one. Int J Biomed Sci 5(4):359–368
24. Vijayabaskar P, Vaseela N (2012) In vitro antioxidant properties of sulfated polysaccharide from brown marine algae *Sargassum tenerrimum*. Asian Pac J Trop Dis 83(5):356–368
25. Wijesinghe WAJP, Jeon YJ (2012) Enzyme-assistant extraction (EAE) of bioactive components: a useful approach for recovery of industrially important metabolites from seaweeds—a review. Fitoterapia 83(2):6–12
26. Yaich H, Garna H, Besbes S, Paquot M, Blecker C, Attia H (2013) Effect of extraction conditions on the yield and purity of ulvan extracted from Ulva lactuca. Food Hydrocoll 31(2):375–382
27. Yilmaz AD, Shirinzadeh H, Coban T, Suzen S, Ozden S (2012) Synthesis and antioxidant activity evaluations of melatonin based analogue indole-hydrazide/ hydrazone derivatives. Int Conf Healthc Med Stud 53(2):058–093
28. Yuling Y, Dan L, Jun W, Yan C, Shusheng W (2011) In vitro antioxidant activities of sulfated polysaccharide fractions extracted from Corallina officinalis. Int J Biol Macromol 49(5):1031–1037